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# Single-Dose Replication-Defective VSV-based Nipah Virus Vaccines Provide Protection from Lethal Challenge in Syrian Hamsters

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## Abstract

Nipah virus (NiV) continues to cause outbreaks of fatal human encephalitis due to spillover from its bat reservoir. We determined that a single dose of replication-defective vesicular stomatitis virus (VSV)-based vaccine vectors expressing either the NiV fusion (F) or attachment (G) glycoproteins protected hamsters from over 1000 times $LD_{50}$ NiV challenge. This highly effective single-dose protection coupled with an enhanced safety profile makes these candidates ideal for potential use in livestock and humans.

### Keywords

Henipavirus; Nipah; vaccine; VSV; single-dose; hamster; glycoproteins

Nipah virus (NiV) is a highly pathogenic paramyxovirus responsible for causing fatal human encephalitis with high case fatality rates from 40%–75% (Lo and Rota, 2008). Since its initial outbreak in Malaysia from 1998–1999, NiV has caused smaller sporadic outbreaks of fatal encephalitis in Bangladesh on a near-annual basis (Luby and Gurley, 2012). A soluble subunit glycoprotein vaccine approved for animal use against the closely-related Hendra virus requiring a two-dose prime-boost regimen has shown protection against NiV in several animal models (Bossart et al., 2012; Broder et al., 2013; McEachern et al., 2008; Mungall et al., 2006; Pallister et al., 2013). Previous work demonstrated that a single dose of replication-defective single-cycle recombinant vesicular stomatitis viruses (VSV-ΔG) expressing either the NiV fusion (F) (VSV-ΔG-NiVF) or attachment (G) (VSV-ΔG-NiVG) glycoproteins induced neutralizing antibodies in mice against VSV-ΔG-particles pseudotyped with NiV F and G glycoproteins (VSV-ΔG-eGFP-NEUT) (Chattopadhyay and

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Rose, 2011). In order to evaluate the protective efficacy of the VSV-ΔG-NiVF and VSV-ΔG-NiVG vaccines against lethal NiV challenge in an animal model that mimics NiV disease, we tested these vaccines in the Syrian golden hamster (DeBuysscher et al., 2013; Guillaume et al., 2004; Rockx et al., 2011; Wong et al., 2003). We obtained approval for animal experiments from the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee (IACUC). All animal work was performed by certified staff in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved biosafety level 2(BSL-2) (vaccination phase) or BSL-4 (challenge phase) facilities at CDC.

We produced stocks of the single-cycle viruses VSV-ΔG-NiVG, VSV-ΔG-NiVF, VSV-ΔG-eGFP pseudotyped with VSV G glycoprotein, and also the VSV-ΔG-eGFP-NEUT pseudotyped with NiV F and G as previously described (Chattopadhyay and Rose, 2011). For vaccination 6-week old female Syrian golden hamsters (*Mesocricetus auratus*, Charles River Laboratories, Wilmington, VA) were anesthetized (isoflurane) and inoculated intramuscularly in the right quadriceps with 1 x $10^6$ infectious particles of either VSV-ΔG-NiVG (10 animals), VSV-ΔG-NiVF (10 animals), or VSV-ΔG-eGFP (10 animals). At 28 days post-vaccination, ~ 100 μl of blood was collected for determination of serum neutralizing antibody titers (SNT) as previously described (Chattopadhyay and Rose, 2011). The vaccinated hamsters, along with 3 additional unvaccinated hamsters (to serve as unvaccinated controls) were transferred into the BSL-4 lab and were given 3 days to adjust to their new surroundings. On day 32 post-vaccination (challenge day 0), all hamsters were inoculated via the intraperitoneal route with a previously described uniformly lethal challenge dose ($10^5$ $TCID_{50}$/hamster, >1000 times $LD_{50}$) of NiV Malaysia strain passaged 3 times on Vero E6 cells (Chua et al., 2000; DeBuysscher et al., 2013; Harcourt et al., 2000; Rockx et al., 2011). Animals were examined and scored daily for two weeks post-challenge for signs of clinical illness, neurologic disease, respiratory distress, and weight loss (weight evaluation for 3 vaccinated groups began on day 3 post-challenge). Animals showing significant weight loss (>25% of initial weight on challenge day 0) alongside any neurological or respiratory signs were humanely euthanized. Animals without clinical illness after 14 days post-infection (p.i.) continued to be monitored daily but were only weighed in 2–5 day intervals until day 32 p.i. in which all surviving animals were humanely euthanized. At time of euthanasia, ~ 3 ml of blood was collected by cardiac puncture for SNT determination. Necropsies were performed to collect lung, spleen, kidney, and brain tissues. Tissues were either inactivated in MAGMAX RNA lysis buffer (Life Technologies, Carlsbad, CA) for subsequent RNA extraction and real-time RT-PCR as previously described (Lo et al., 2012), or fixed in 10% formalin for histopathology and immunohistochemistry (IHC) analysis as previously described (Wong et al., 2003).

On day 6 post-challenge, all unvaccinated control hamsters either died or were euthanized due to the development of neurologic signs and respiratory distress. Similarly, 5 out of 10 (50%) backbone control VSV-ΔG-eGFP-vaccinated hamsters either died or were euthanized due to the onset of illness. By day 9 post-challenge, all VSV-ΔG-eGFP-vaccinated hamsters had either died or were euthanized (Figure 1A). In contrast, hamsters vaccinated with VSV-ΔG-NiVG or VSV-ΔG-NiVF did not develop any clinical illness nor weight loss throughout the course of infection, and were euthanized on day 32 p.i. (day 64 post-vaccination) (Figure 1A, 1B). SNTs against NiV were defined as the reciprocal of the highest serum dilution at which duplicate wells of each serum sample showed complete neutralization of 50 infectious particles of VSV-ΔG-eGFP-NEUT. Prior to NiV challenge, hamsters vaccinated with either VSV-ΔG-NiVG or VSV-ΔG-NiVF developed respective SNTs of 640 and 160, while hamsters vaccinated with VSV-ΔG-eGFP did not develop any detectable SNT (Figure 2A). Following NiV challenge, both unvaccinated control and VSV-ΔG-eGFP-vaccinated groups developed low levels of neutralizing antibodies (Figure 2B), but even at serum dilutions of

1:20 could not completely neutralize 50 particles of VSV-ΔG-eGFP-NEUT. The lack of an anamnestic immune response in the VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated groups following NiV challenge possibly indicates sterilizing immunity, as their respective post-challenge SNTs at the time of euthanasia remained similar to pre-challenge levels with comparatively lower percentages of neutralization at higher serum dilutions of 1:2560, 1:5120 and 1:10240 (Figure 2A, 2B). Histopathology and IHC results in unvaccinated and VSV-ΔG-eGFP-vaccinated hamsters were similar to those observed in a recent study of NiV pathogenesis in the hamster model (DeBuysscher et al., 2013), indicating bronchointerstitial pneumonia with vasculitis associated with virus replication (Figure 3). In the unvaccinated and eGFP control-vaccinated groups we detected both viral RNA and viral antigen in all tissues sampled from both groups except for the brain, in which we detected viral RNA but not viral antigen (Table 1, Figure 3, data not shown). In contrast, we did not observe any pathology and could neither detect the presence of viral RNA nor antigen in any tissues collected from VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated groups (Table 1, data not shown).

In summary, we have demonstrated that a single dose of these single-cycle vaccine candidates expressing either NiV G or F conferred complete protection from lethal NiV challenge in the Syrian golden hamster animal model. This is in marked contrast with all other previously evaluated viral vaccine vectors expressing NiV G or F that required multi-dose prime-boost regimens (Guillaume et al., 2004; Weingartl et al., 2006; Yoneda et al., 2013) with the exception of an adenovirus-associated virus (AAV) vector which required an extremely high dose of vaccine ($6 \times 10^{11}$ genome particles) for protection (Ploquin et al., 2013). Furthermore, when compared to these other vectors expressing NiV G, the VSV-ΔG-NiVG induced higher levels of SNT at 28 days post-vaccination, and was the only vaccine administered in which no anamnestic immune response was detected. Additionally, the NiV challenge dose used in this study was at least 10 times greater than that used by any other NiV vaccine protection study in the hamster model, which highlights the extremely robust protection conferred by the VSV-ΔG NiV vaccines. While replication-competent VSV-based vectors expressing appropriate foreign antigens have shown to be highly effective vaccines against a number of viral and bacterial pathogens (Cobleigh et al., 2010; Jones et al., 2005; Kahn et al., 2001; Kapadia et al., 2005; Liao et al., 2008; Palin et al., 2007; Roberts et al., 1998; Roberts et al., 2004; Rose et al., 2001), regulatory approval of these vaccine vectors in humans has been slow due to concerns regarding potential pathogenesis. On the other hand, replication-deficient VSV-ΔG vectors have shown to induce equivalent or even greater levels of humoral and cell-mediated immunity when compared with its corresponding replication-competent vector, particularly when vaccinating via the intramuscular route (Kapadia et al., 2008; Publicover et al., 2005). In comparison to the soluble HeV G subunit vaccine (HeVsG) currently in animal trials (Broder et al., 2013), the VSV-ΔG vector provides some advantages. The first and foremost advantage is the single-dose requirement for protection, and the second advantage is the lack of any adjuvants required to generate robust humoral and cell-mediated T-helper 1 (Th1) type immunity (Publicover et al., 2005). The two-dose HeVsG vaccine formulation requires the action of two adjuvants, Allhydrogel and CpG oligodeoxynucleotide (ODN) 2006 (Bossart et al., 2012) in order to generate a Th1 immune response, since Allhydrogel alone typically induces a Th2 response which is not appropriate against viral infections (Coffman et al., 2010; Steinhagen et al., 2011; Weeratna et al., 2001). The HeVsG vaccine however holds an advantage over the VSV-ΔG vector in that the vaccine antigen is more easily produced on a large scale versus the production of infectious VSV-ΔG particles which require multiple plasmid transfections (Pallister et al., 2011; Witko et al., 2010). The results reported in this study underscore the safety profile and robust protection conferred by a single dose of these replication-deficient single-cycle vectors against NiV. Future studies will establish the

minimum protective dose and early protection kinetics of the VSV-ΔG NiV vaccines as potential tools for prophylaxis and reduction of NiV disease severity and/or mortality.

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## Highlights

- A single dose of VSVΔG-based vaccines expressing Nipah glycoproteins protected hamsters.
- VSVΔG-based NiV vaccines induced high levels of serum neutralizing antibodies with one dose.
- Lack of detectable anamnestic immune responses indicates potential sterilizing immunity.

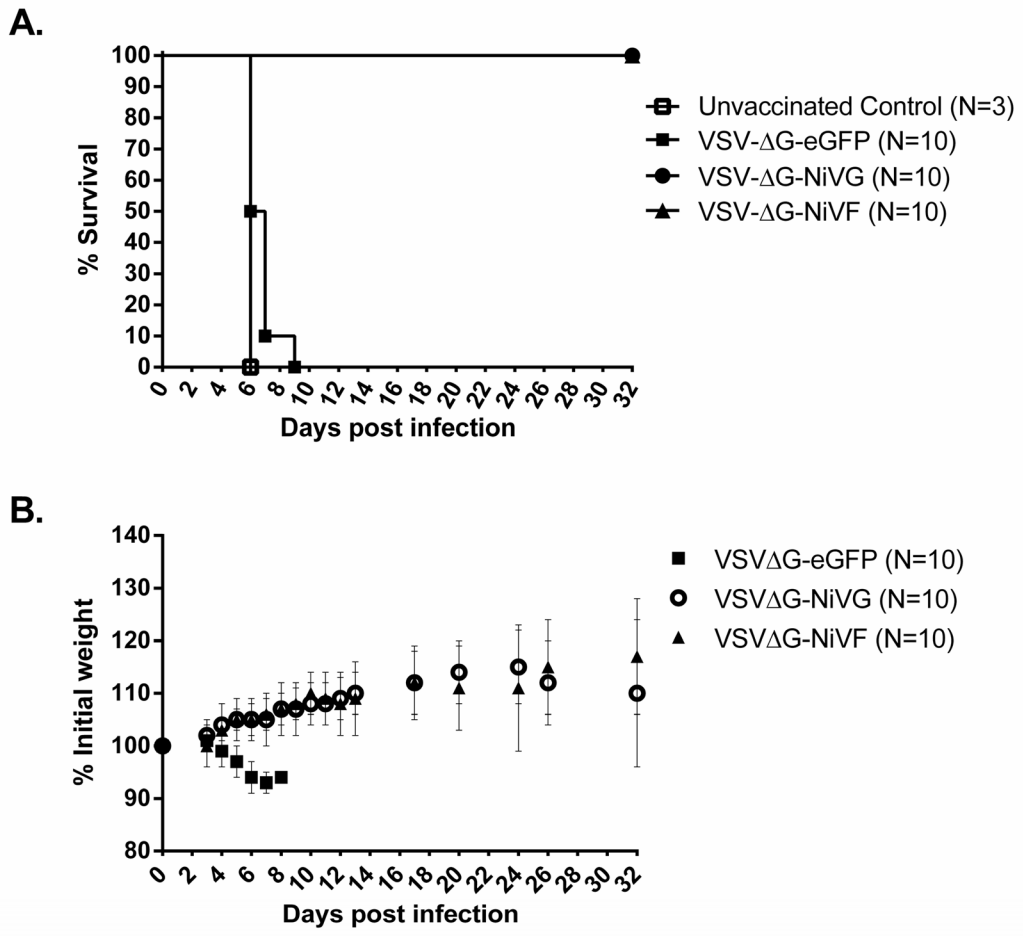


**Figure 1.**

A single-dose vaccination of hamsters with single-cycle replication-deficient VSV viral vectors (VSV-ΔG) expressing either NiV G or NiV F confers complete protection from lethal NiV challenge. **(A)** Survival curves of unvaccinated hamsters and hamsters vaccinated with VSV-ΔG-NiVG, VSV-ΔG-NiVF, or VSV-ΔG-eGFP, and challenged 32 days later with virulent NiV. **(B)** Weight curves of vaccinated hamsters challenged with lethal dose of NiV. Weight changes are expressed as the mean percentage changes for NiV challenged animals relative to their weights at day zero.

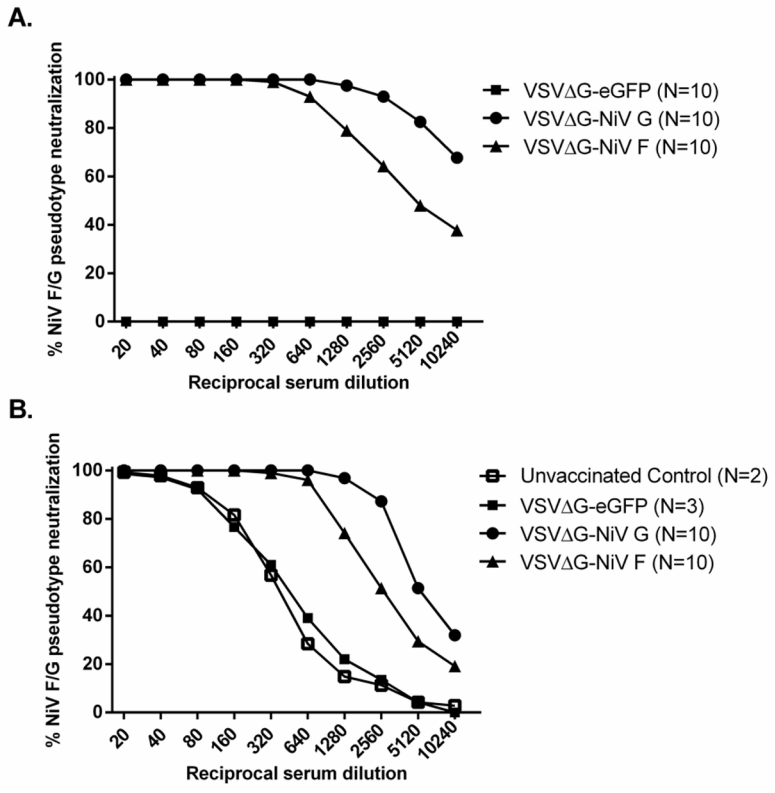


**Figure 2.**

A single-dose vaccination of hamsters with either VSV-ΔG-NiVG or VSV-ΔG-NiVF induces protective serum neutralizing antibody titers (SNT). Percent neutralization curves of pooled serum from each group of hamsters at **(A)** 28 days post vaccination pre-NiV challenge, and **(B)** 64 days post-vaccination, 32 days post-NiV challenge for VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated hamsters, 6 days post-challenge for unvaccinated hamsters, and at 6, 7, and 9 days post-challenge for VSV-ΔG-eGFP-vaccinated hamsters. SNT for each group was determined as the highest reciprocal serum dilution in which 50 particles of VSV-ΔG-eGFP virus pseudotyped with NiV F and G glycoproteins were completely (100%) neutralized.

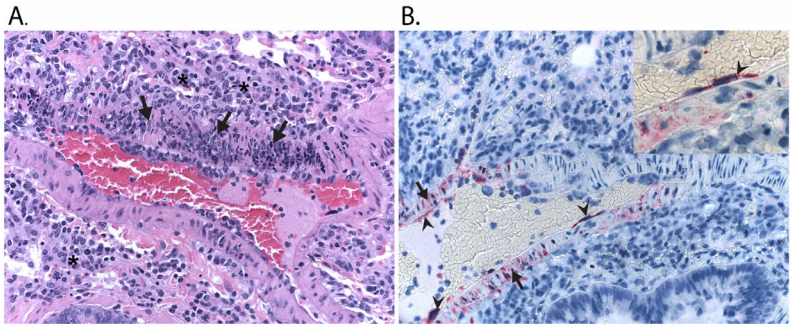


**Figure 3.**

Lungs from unvaccinated hamsters show vasculitis and abundant Nipah virus antigen associated with bronchial arteries. **(A)** Vasculitis characterized by acute inflammation and necrotic cellular debris that obscures the arterial wall (arrows). The adjacent parenchyma shows interstitial pneumonia (asterisks). **(B)** Nipah virus IHC shows abundant antigen (red) in endothelial cells (arrow heads) and smooth muscle cells of artery (arrows). **Inset**: Nipah virus antigen within endothelial cell (arrow head). Original magnification: 100x, 400x (inset).

## TABLE 1

Mean NiV nucleoprotein gene RNA copy numbers/μg RNA from tissue

| ***Tissue*** | | | | |
|---|---|---|---|---|
| | **Unvaccinated Control (N=3)** | **VSV-ΔG-eGFP (N=10)** | **VSV-ΔG-NiVG (N=10)** | **VSV-ΔG-NiVF (N=10)** |
| Spleen | 4.26E+06 | 1.66E+06 | ND | ND |
| Kidney | 3.56E+06 | 1.63E+06 | ND | ND |
| Lung | 9.68E+05 | 7.35E+06 | ND | ND |
| Brain | 2.70E+05 | 2.70E+05 | ND | ND |

ND= not detected